Thin-Layer (Planar) Chromatography

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Introduction

In chiral chromatography, two diastereomeric adducts with different physicochemical properties are formed during elution. The adducts differ in their stability (in chiral stationary phases, CSPs, or chiral coated phases, CCPs) and/or in their interphase distribution ratio (in chiral mobile phases, CMPs).

According to Dalgliesh, three active positions of the selector must interact simultaneously with the active positions of the enantiomer to reveal differences between optical antipodes. This is a sufficient condition for resolution to occur but it is not essential. Chiral discrimination may happen as a result of hydrogen bonding and steric interactions, making only one attractive force necessary in this type of chromatography. Moreover, the creation of specific chiral cavities in a polymer network (as in 'molecular imprinting' techniques) could make it possible to base enantiomeric separations entirely on steric fit.

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Chiral Stationary Phases and Chiral Coated Phases

Few chiral phases are used in TLC; one of the main reasons for this is that chiral stationary phases with very high UV background can only be used only with fluorescent or coloured solutes. For example, amino-modified ready-to-use layers bonded or coated with Pirkle-type selectors, such as *N*-(3,5-dinitrobenzoyl)-L-leucine or *R*(−)-α-phenylglycine, are pale yellow and strongly adsorb UV light. Another reason is the high price of most CSPs.

The most widely used CSPs or CCPs are polysaccharides and their derivatives and silanized silica gel impregnated with an optically active copper (II) complex of derivatized hydroxyproline. The use of silica gel impregnated with chiral polar selectors, such as D-galacturonic acid, (+)-tartaric acid, (−)-brucine, L-aspartic acid or a complex of copper (II) with L-proline, should also be mentioned.

In CSPs, owing to the nature of the polymeric structure, the simultaneous participation of several chiral sites or several polymeric chains is conceivable. In CCPs, the chiral sites are distributed at the surface or in the network of the achiral matrix relatively far away from each other and only a bimolecular interaction is generally possible with the optical antipodes to be separated.

Cellulose

The linear polysaccharide cellulose is composed of (+)-D-glucose units and its relative molecular mass ranges from 2.5×10^5 to 1×10^6 or more. The long chains are arranged on a partially crystalline fibre structure and held together by numerous hydrogen bonds between the hydroxyl groups. The hydrolysis of cellulose with 2.5 mol L^{-1} hydrochloric acid at *c.* 100°C removes amorphous material and yields a more crystalline polymer called 'microcrystalline cellulose' and marketed as Avicel by several companies.

The mechanism of chiral recognition is not yet completely clarified even though a significant role is attributed to the cellulose structure and to the hydroxyl groups, the protection of which with BrCN resulted in the loss of chiral recognition. The optical antipodes resolved are highly polar, such as amino acids, with multiple sites for hydrogen bond formation.

Cellulose Derivatives

Among derivatized polysaccharides, cellulose triacetate (CTA) is the most used stationary phase for the resolution of racemic compounds by TLC.

The different fit of the two enantiomers into the laminae of the polymer leads to separation of the optical antipodes which is mainly governed by the shape of the solutes (flat molecules showing a better permeation into the cavities) and only to a minor extent by electrostatic interactions involving the functional groups of the molecules. Hence the name 'inclusion chromatography'. In addition, the chiral recognition of CTA depends strongly on its structure and the type of eluent and increases as the crystallinity of the polysaccharide is increased. Microcrystalline cellulose triacetate (MCTA) can be prepared from microcrystalline cellulose with almost complete preservation of microcrystallinity. Usually, the type of eluent and its composition are important for chiral recognition because these produce different swelling of MCTA, which in turns enables the separation of solutes of different sizes and characteristics.

The use of *n*-hexane–isopropanol mixtures resulted in unsatisfactory separations since extremely elongated spots are generally obtained. Aqueous–alcoholic solutions have the opposite effect, giving rise to round and compact spots.

This CSP is able to resolve a broad range of structurally different racemates. In general, more polar molecules require a higher percentage of water than hydrophobic compounds.

Hydrophobic Silica Gel Impregnated with Copper (II) Complex of (2*S*,4*R*,2′*RS*)-*N*-(2′-hydroxydodecyl)-4-hydroxyproline

The structure of the selector is shown in **Figure 1**. Chiralplate and HPTLC-CHIR are the only precoated plates built up from such material. The chiral layer on the latter plates is combined with a so-called 'concentrating zone'. The sample to be separated is applied to this small band and is transported with the solvent front, forming a narrow band at the interface of the two sections; consequently, a higher efficiency of the separation process is obtained.

Many racemates have been resolved on both layers by ligand-exchange chromatography (LEC). The separated enantiomers are those capable of forming diastereomeric complexes of different stability with the metal ion and the chiral selector. The requirement of sufficient stability of the ternary complex involves five-membered ring formation and compounds such as α-amino and α-hydroxy acids are the most suitable.

Chiral Mobile Phases

CMPs permit the use of conventional stationary phases and show fewer detection problems than CSPs or CCPs. However, high cost chiral selectors (i.e. γ-cyclodextrin) are certainly not advisable for TLC.

Enantiomer separations have been achieved using chiral mobile phases in both normal and reversed-phase chromatography. The first technique employs silica gel and, mostly, Diol F_{254} HPTLC plates (Merck) and, as chiral selectors, D-galacturonic acid, *N*-carbobenzoxy(CBZ)-L-amino acids or peptides, 1*R*-(−)-ammonium-10-camphorsulfonate and 2-*O*-[(*R*)-2-hydroxypropyl)]-β-cyclodextrin.

Most separations have been obtained by reversed-phase chromatography on hydrophobic silica gel with β-cyclodextrin (β-CD) and its derivatives, bovine serum albumin (BSA) and the macrocyclic antibiotic vancomycin as chiral agents.

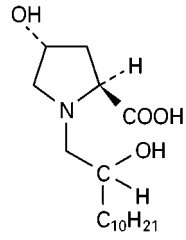

Figure 1 Structure of (2*S*,4*R*,2*RS*)-*N*-(2-hydroxydodecyl)-4-hydroxyproline.

Unmodified and Modified β-Cyclodextrins as Mobile-Phase Additives

Among the three cyclodextrins (α, β, γ), only β-CD and its derivatives have been used for successful resolution of various racemates by TLC. In an aqueous solution β-CD is represented as a truncated cone with different sized mouths (0.60–0.65 nm); the height of the cavity is 0.78 nm. The 2- and 3-hydroxyl groups are oriented towards the outside and are responsible for the aqueous solubility properties of this oligosaccharide. The hydrogen atoms and glycosidic oxygen groups are located inside the molecule, forming the relatively hydrophobic cavity that interacts with organic optical isomers to form diastereomeric inclusion complexes. Under reversed-phase conditions, the combination of hydrophobic and steric interactions with hydrogen bonding between the chiral solutes and the 2- and/or 3-hydroxyl groups may be the cause of enantioselectivity.

Aqueous–organic solutions (i.e. methanol–water or acetonitrile–water mixtures) are usually used as eluents. β-CD is enantioselective in TLC only at the high concentrations reached by adding large amounts of urea, which increases the aqueous solubility of β-CD more than ten-fold. However, urea tends to compete with solutes for the preferred location in the hydrophobic cavity, thus decreasing the separation factor. Chemical modification with hydroxypropyl, hydroxyethyl or methyl groups has been used to increase the solubility of β-CD and its complexes in water, eliminating the need to use urea. Optimization of the enantioselectivity can be achieved by modifying the concentration and nature of organic solvent, pH and buffer concentration of the eluent.

Bovine Serum Albumin as Mobile-Phase Modifier

BSA is a protein of relative molecular mass 66 210, consisting of 581 amino acids in a single chain. It is a relatively acidic protein (isoelectric point 4.7), highly soluble in water, but precipitates from high salt solutions. At pH 7.0 its net charge is −18. Hydrophobic interactions strongly contribute to the affinity of organic solutes for BSA; simultaneous contributions exist from electrostatic interactions, steric effects, hydrogen bonding and charge-transfer processes.

Mobile phases containing this chiral selector have been employed for the resolution of a broad variety of racemic compounds on silanized silica plates. Eluents were prepared by dissolving BSA (Serva, Heidelberg,

FRG), fraction V, pH 5.2 or 7.0, in different buffer systems and then adding the desired amount of 2-propanol. The results suggest the use of acidic eluents to separate *N*-derivatized amino acids on wettable RP-18W/UV_{254} (Macherey-Nagel) or RP-18W/F_{254} (Merck) plates. On the other hand, the resolution of free amino acids increases with increasing eluent pH; in particular, the useful pH range on SIL C_{18}-50/UV_{254} layers (Macherey-Nagel) is 9–10.

A prerequisite for optical discrimination is the presence of aromatic as well as polar groups in the solute. The existence of stereospecific binding sites on albumin is well known (i.e. tryptophan and warfarin) and it is believed that this binding occurs at a number of relatively defined regions. Two independent noncooperative types of sites (chiral and achiral) coexist on the protein; the retention mechanism is partially in accord with that proposed on columns packed with immobilized BSA since in TLC albumin is used as a mobile-phase additive.

Retention and Resolution Data

In this article the contribution of chiral TLC to enantiomeric separations is surveyed, emphasizing the versatility of the method but without discussing the chiral recognition mechanism; this aspect has been examined elsewhere and in other parts of this Encyclopedia.

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Amino Acids and Their Derivatives

A broad variety of racemic amino acids has already been resolved by chiral TLC. **Table 1** summarizes the analytical separations achieved in this field on 20 cm × 20 cm Chiralplates (Cat. No. 811058, Macherey-Nagel; thickness 0.25 mm). The separations can be easily transferred to the 10 cm × 10 cm HPTLC-CHIR plates with concentrating zone since they are precoated with the same chiral selector.

With eluents A, B and C, 2 μL of a 1% solution of the racemates in methanol or methanol–water and with eluent D, 2 μL of a 0.5% solution of the racemates in 0.1 mol L^{-1} hydrochloric acid–methanol 1 : 1 were applied to the plates. Migration time increases from 0.5 h (eluent A) to 1.5 h (eluent C). Detection was performed by dipping the plates for 3 s in a 0.3% ninhydrin solution in acetone and then heating at 110°C for *c.* 5 min. Red spots appear on a white background.

The amount of solute applied to the plates (10–20 μg) is an order of magnitude greater than that generally employed in TLC; the use of HPTLC-CHIR layers improves the sensitivity of the method.

Thus far, 84 proteinogenic and nonproteinogenic amino acids have been separated without derivatization using mostly methanol–water–acetonitrile (50 : 50 : 200, v/v/v) as eluent. Usually the D enantiomer is the more retained. Racemic serine shows low resolution, while threonine and basic amino acids have not been resolved as yet.

The separations of enantiomeric amino acids reported in **Table 2** using a variety of chiral selectors are very interesting since they also include the unresolved compounds mentioned above. Round, compact spots are generally obtained on silica gel plates eluted with 2-*O*-[(*R*)-2-hydroxypropyl]-*β*-cyclodextrin solutions as deduced from R_s values which are equal to or higher than the α value for all the amino acids with the only exception of DL-citrulline ($R_s = 0.94$). Visualization is performed by spraying the plates with a salicyladehyde solution (1.5 g) in 100 mL toluene and then heating at 50°C for 10 min (yellow spots).

Table 3 gives the performance of cellulose plates, which are very effective in resolving racemates of aromatic and basic amino acids. Home-made layers of microcrystalline cellulose powder (commercially available from Merck, and Fluka) can be obtained with optimal homogeneity by spreading an aqueous suspension with about 25% chiral material. The plates are dried at room temperature and do not require activation before use. Polar mixtures (i.e. ethanol–pyridine–water) are the best eluents since they separate enantiomers as efficiently as an aqueous solvent (i.e. 0.1 mol L^{-1} NaCl) but give rise to more compact spots.

Chiralplates were very effective in resolving racemates of *N*-alkyl, *N*-carbamyl and *N*-formyl amino acids and of several dipeptides (**Table 4**). It is worth noting that the dipeptide with the *C*-terminal L-configuration always has a higher retention than the one with the *C*-terminal D-configuration. Some racemic dipeptides were also resolved on microcrystalline cellulose with pyridine–water (2 : 1 or 4 : 1) and on SIL C_{18}-50/UV_{254} plates using BSA as chiral mobile phase additive.

Derivatization of amino acids may be used to improve chiral recognition, detectability and sensitivity of the method and to label amino acids residues of peptides and proteins, especially the *N*-terminal amino acid. Dimethylaminonaphthalenesulfonyl (dansyl) amino acids form when primary and secondary amino acids react with dansyl chloride, generating strongly fluorescent compounds. The best chromatographic conditions for their separation are reported in **Table 5**. The most complete study, performed on KC18 F plates (Whatman) using *β*-CD as chiral agent, concerns the racemates of 26

Table 1 Enantiomeric separation of proteinogenic and nonproteinogenic amino acids on Chiralplates[a]

Racemate	hR_{F1}[b]	hR_{F2}	α[c]	Eluent[d]
Ala	69(D)	73	1.22	D
Ser	73(D)	76	1.17	D
Abu	48	52	1.17	A
Val	54(D)	62	1.39	A
Nva	49(D)	56	1.32	A
Leu	53(D)	63	1.51	C
Ile	47(D)	58	1.55	A
Nle	53(D)	62	1.44	A
allo-Ile	51(D)	61	1.50	A
t-Leucine	40(D)	51	1.56	A
Met	54(D)	59	1.23	A
Eth	52(D)	59	1.32	A
Pro	41(D)	47	1.27	A
cis-Hyp[e]	41(L)	59	2.08	A
allo-Hyp	41(L)	59	2.08	A
Pipecolic acid	51	58	1.32	D
Phe	49(D)	59	1.49	A
Homophenylalanine	49(D)	58	1.43	A
Tyr	58(D)	66	1.40	A
Dopa	47(L)	58	1.55	B
Trp	51(D)	61	1.50	A
Asp	50(D)	55	1.22	A
Glu	54(D)	59	1.22	A
Gln	41(L)	55	1.76	A
Thyroxine	38(D)	49	1.56	A
PhenylGly	57(D)	67	1.53	A
CyclopentylGly	43	50	1.32	A
(1-Methylcyclopropyl)-Gly	49	57	1.38	A
(2-Thienyl)-Gly	55	66	1.58	A
3-Cyclopentyl-Ala	46	56	1.49	A
3-(1-Naphthyl)-Ala	49(D)	56	1.32	A
3-(2-Naphthyl)-Ala	44(D)	59	1.82	A
Met(O_2)[f]	62(D)	66	1.18	A
Eth(O_2)	55	59	1.18	A
Seleno-Met	53(D)	61	1.38	A
S-Methylthio-Cys	47(D)	55	1.38	A
S-Methylthio-HomoCys	44	52	1.38	A
S-(2-Chlorobenzyl)-Cys	45	58	1.68	A
S-(3-Thiobutyl)-Cys	53	64	1.58	A
S-(2-Thiopropyl)-Cys	53	64	1.58	A
O-Benzyl-Ser	54(D)	65	1.58	A
O-Benzyl-Tyr	48(D)	64	1.92	A
3,3-Dimethyl-Nva	40(D)	56	1.91	A
4-Methyl-Trp	50	58	1.38	A
5-Methyl-Trp	52	63	1.57	A
6-Methyl-Trp	52	64	1.64	A
7-Methyl-Trp	51	64	1.70	A
4-Methoxy-Phe	52	64	1.64	A
5-Methoxy-Trp	55	66	1.58	A
3-Chloro-Ala	57	64	1.34	A
4-Amino-Phe	33	47	1.80	A
4-Bromo-Phe	44	58	1.75	A
4-Chloro-Phe	46	59	1.68	A
2-Fluoro-Phe	55	61	1.28	A
4-Iodo-Phe	45(D)	61	1.91	A
4-Nitro-Phe	52	61	1.44	A
3-Fluoro-Tyr	64	71	1.37	A
5-Bromo-Trp	46	58	1.61	A
α-Methyl-Ser	56(L)	67	1.59	B
α-Methyl-Abu	50	60	1.50	A
α-Methyl-Val	51	56	1.22	A

Table 1 *Continued*

Racemate	hR_{F1}[b]	hR_{F2}	a[c]	*Eluent*[d]
α-Methyl-Leu	48	59	1.55	A
α-Methyl-Met	56(D)	64	1.39	A
α-Methyl-Phe	53(L)	66	1.72	A
α-Methyl-Tyr	63(D)	70	1.37	A
α-Methyl-Dopa	46(L)	66	2.27	B
α-Methyl-Trp	54	65	1.58	A
α-Methyl-Asp	52(D)	56	1.17	A
α-Methyl–Glu	58(L)	62	1.18	A
α-Ethyl-Ala	55	61	1.28	A
α-Propyl-Ala	55	63	1.39	A
α-Butyl-Ala	51	63	1.63	A
α-Difluoromethyl-Phe	63	70	1.37	A
α-Propenyl-Phe	57	63	1.28	A
2′-Methyl-Phe	43(D)	54	1.55	A
β-Methyl-Phe	36(*R*, *R*)	56(*S*, *S*)	2.26	A
2′-Methyl-β-methyl-Phe	48(*S*, *R*)	55(*R*, *S*)	1.32	A
2′-6′-Dimethyl-Phe	38(D)	52	1.76	A
β-Methyl-p-nitro-Phe	43(*R*, *R*)	62(*S*, *S*)	2.16	A
	52(*S*, *R*)	60(*R*, *S*)	1.38	A
2′,5′-Dimethyl-4-methoxy-Phe	45(D)	57	1.61	A
β-Hydroxy-Phe	49(*R*, *R*)	63(*S*, *S*)	1.77	A
β-Methyl-Tyr	52(*R*, *R*)	67(*S*, *S*)	1.87	A
	55(*S*, *R*)	67(*R*, *S*)	1.66	A
2′-Methyl-Tyr	54(D)	62	1.39	A
2′,5′-Dimethyl-Tyr	56(D)	67	1.59	A

[a] Migration distance 13 cm; chamber saturation.
[b] $hR_F = R_F \times 100$.
[c] $\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.
[d] A = methanol–water–acetonitrile 50 : 50 : 200 (v/v/v); B = methanol–water–acetonitrile 50 : 50 : 30 (v/v/v); C = methanol–water 10 : 80 (v/v); D = acetone–methanol–water 10 : 2 : 2 (v/v/v).
[e] Hyp = hydroxyproline.
[f] Met(O_2) = methionine sulfone.

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common and uncommon dansyl (Dns) amino acids. Similar results can be obtained on 10 cm × 10 cm SIL C_{18}-50/UV_{254} layers with the same eluents but with lower migration times. Some enantiomeric Dns-amino acids such as Lys, Met, Nva, Pro and aromatic compounds show low selectivity coefficients with β-CD. Therefore, it can be useful to resolve these racemates on RP-18W/UV_{254} plates with eluents containing BSA since very high α values have been achieved.

Other *N*- and *C*-terminal substituents studied by chiral TLC include 2,4-dinitrophenyl (DNP), 3,5-dinitro-2-pyridyl (DNPy), 3,5-dinitrobenzoyl (DNB), *o*-nitrophenylsulfenyl (*o*-NPS), 9-fluorenylmethoxycarbonyl (FMOC), methylthiohydanthoin (MTH), phenylthiohydanthoin (PTH), *t*-butoxycarbonyl (*t*-BOC), carbobenzoxy (CBZ), phthalyl, acetyl, *p*-nitroanilide (pNA) and β-naphthylamide (βNA) (**Table 6**).

The maximum ΔR_F for the enantiomers of FMOC amino acids was obtained at different concentrations of 2-propanol. It is worth noting that this is the first time optical isomers have been separated with eluents containing BSA in the presence of very high levels (12–36%) of organic modifier. The resulting spots have the shape of a reversed triangle. FMOC-DL-Asn and FMOC-DL-Gln are not resolved. The order of retention of the D and L forms of the different compounds is variable. The D forms of FMOC-Pro, FMOC-Trp and FMOC-Met are more retained than the L forms, whereas the opposite is noted for the other amino acids. This behaviour is also shown from DNP-amino acids and other *N*-derivatives.

Most DNP, DNPy and DNB-DL-amino acids are resolved on RP-18W/UV_{254} plates with 0.1 mol L^{-1} acetate buffer solutions containing 2% isopropanol and different BSA concentrations (2–6%) but few of them show chiral separation with phosphate buffer (0.05 mol L^{-1} potassium dihydrogen phosphate + 0.05 mol L^{-1} disodium hydrogen phosphate), an eluent of higher pH (6.86) than that previously used. Enantiomeric DNPy-Ala, DNPy-Nva and DNP-Eth(O_2) are completely separated at low

Table 2 Resolution of racemic amino acids by chiral TLC

Racemate	hR_{F1} [a]	hR_{F2}	α [b]	*Separation technique*
Ala	18(D)	53	5.13	Slurry of silica gel (Merck) and (−)-brucine brought to pH 7.1 with 0.1 mol L^{-1} NaOH and spread on 20 cm × 20 cm plates. Eluent: butanol–acetic acid–water 3 : 1 : 4 (v/v/v). Migration distance, 10 cm; development time 0.5 h. Visualization: ninhydrin.
Ser	12(D)	50	7.33	
Thr	16(D)	29	2.15	
Ile	16(D)	35	2.82	
Met	18(D)	29	1.86	
Phe	27(D)	40	1.80	
Tyr	22(D)	29	1.45	
Trp	17(D)	31	2.19	
Trp	59(D)	72	1.77	SIL C_{18}-50/UV_{254} plates (Cat. No. 711308, MN) 10 cm × 10 cm, thickness 0.20 mm. Eluent: 0.05 mol L^{-1} $NaHCO_3$ + 0.05 mol L^{-1} Na_2CO_3 containing 6% BSA and 6% isopropanol (pH 9.8); for the resolution of 7-methylTrp, 5-methoxyTrp and Kynurenine, 0.05 mol L^{-1} sodium tetraborate was used. Migration distance, 8 cm; development time 1 h 50 min. Visualization: *p*-dimethylaminobenzaldehyde.
Trp-NH_2 [c]	31(L)	40	1.48	
4-Methyl-Trp	42	65	2.56	
5-Methyl-Trp	37	61	2.53	
6-Methyl-Trp	66	78	1.82	
7-Methyl-Trp	41	50	1.43	
5-Methoxy-Trp	42	49	1.32	
4-Fluoro-Trp	51	66	1.86	
5-Fluoro-Trp	43	63	2.23	
6-Fluoro-Trp	42	54	1.62	
Kynurenine	69(D)	80	1.80	
3-(1-Naphthyl)-Ala	34(D)	40	1.29	
Val	62(D)	68	1.30	DC plastikfolien, Kieselgel 60 F_{254} (Merck), 20 cm × 20 cm, thickness 0.2 mm. Eluent: acetonitrile–water 1 : 2.5 for Arg, His and Lys and 1.5 : 2 for the others; the water containing $6.5 \cdot 10^{-3}$ mol L^{-1} 2-*O*-[(*R*)-2-hydroxypropyl]-β-CD. Migration distance, 18 cm at 19°C.
Gln	59(D)	66	1.35	
Arg	50(D)	59	1.44	
Cit	65(D)	69	1.20	
His	46(D)	55	1.43	
Lys	49(D)	60	1.56	

[a] $hR_F = R_F \times 100$.
[b] $\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.
[c] Tryptophanamide.

temperature (10°C) and pH values (0.5 mol L^{-1} acetic acid) where their retention by the layer is sufficient. The unresolved racemates include DNPy-Ser, DNP-Asp, DNP-Glu and DNPy-Trp. The first three amino acids are markedly polar and are only slightly retained by silanized silica gel plates, even when eluted with acidic solution; this may be the reason for their not being resolved. In general, planar chromatography clearly separates the enantiomers of *N*-derivatized hydrophobic amino acids.

The complete resolution of DNPy-DL-Trp is obtained on layers of SIL C_{18}-50/UV_{254} with β-CD as chiral agent.

The optical isomers of PTH-amino acids are sensitive to light and readily racemize. Racemization of these optical active derivatives is observed on silanized silica gel plates with acidic eluents. MCTA plates may be useful since they are able to separate enantiomeric MTH-Phe, MTH-Tyr, MTH-Pro and PTH-Pro with neutral aqueous–alcoholic eluents.

Among C-terminal substituents, the enantiomeric βNA derivatives of amino acids were well separated on silanized silica gel plates with β-CD as mobile phase modifier while pNA derivatives show discordant results. In fact, DL-Leu-pNA is fully resolved but DL-Ala-pNA failed since the latter optical antipodes do not form inclusion complexes of sufficient stability with β-CD. In addition, BSA seems efficient in the enantioseparation of pNA derivatives.

α-Hydroxycarboxylic Acids

Table 7 reports the separation and resolution data for aliphatic and aromatic DL-α-hydroxycarboxylic acids on HPTLC-CHIR plates with concentrating zone where the selectivity coefficients appear to be higher than those obtained on Chiralplates using the same eluent (dichloromethane–methanol, 45 : 5 v/v). Vanadium pentoxide may be used for detection of aromatic and aliphatic compounds. The oxide (1.82 g) is dissolved in 30 mL of 1 mol L^{-1} sodium carbonate by ultrasonic bath and, after cooling, 46 mL of 2.5 mol L^{-1} sulfuric acid and acetonitrile to 100 mL are added. Plates dipped in this solution and allowed to stand at room temperature give blue spots on a yellow background. All racemates studied were

Table 3 Retention and resolution data for racemic amino acids on home-made and precoated cellulose plates

Racemate	hR_{F1}[a]	hR_{F2}	α[b]	*Eluent*	*Remarks*
Trp	50(D)	53	1.12	A	Home-made microcrystalline cellulose plates (Avicel SF, Funakoshi, Japan) 20 cm × 20 cm. Development time 2.3 h; visualization: UV_{365}. A = methanol–butanol–benzene–water 2 : 1 : 1 : 1 (v/v/v/v).
5-HydroxyTrp	25(D)	31	1.35	A	
Kynurenine	54(D)	61	1.33	A	
3-Hydroxykynurenine	47(D)	53	1.27	A	
5-Hydroxykynurenine	20(D)	26	1.40	A	
3-Methoxykynurenine	55(D)	62	1.33	A	
N-α-acetylkynurenine	74(D)	82	1.60	A	
Diaminoadipic acid	23(D, D)	28	1.30	B	Cellulose plates (Merck); B = methanol–water–acetic acid 40 : 10 : 2 (v/v/v).
Diaminopimelic acid	25(D, D)	37	1.76	B	
Trp	46(L)	52	1.27	C	DC-Alufolien Cellulose F_{254} plates (Merck) (20 cm × 20 cm × 0.1 mm). Development time 1.8–4.5 h; migration distance 15 cm; visualization: ninhydrin. Eluents: methanol–water 3 : 1 (D), 7 : 3 (F), 3 : 2 (C); *n*-butanol–acetic acid–water 1 : 1 : 1 (E). Layers heated at 110°C for 5 min before use.
5-HydroxyTrp	34(L)	41	1.34	C	
Kynurenine	38(D)	47	1.44	C	
4-AminoPhe	40(L)	45	1.22	D	
Phe-4-sulfonic acid	70(L)	73	1.15	E[c]	
o-Tyr	57	61	1.17	F	
m-Tyr	55	59	1.17	F	
p-Tyr	81(L)	83	1.14	F[c]	
p-Tyr-3-sulfonic acid	30(L)	40	1.55	E	
Dopa	53(L)	57	1.17	C	
Trp	40(L)	49	1.44	G	Avicel SF plates 20 cm × 20 cm, Lot 8390, Funakoshi, Japan. Development time 11.5 h at 0°C. Visualization: ninhydrin. G = ethanol–pyridine–water 2 : 3 : 1 (v/v/v) or 1 : 1 : 1 (v/v/v).
His	11(D)	13	1.20	G	
Phe	55(L)	59	1.17	G	
Tyr	53(L)	60	1.33	G	
Dopa	43(L)	50	1.32	G	
Cys	6(D)	8	1.35	G	
Thr	51(D)	56	1.22	G	
Tyr	75(L)	81	1.42	H	DC-Plastikfolien Cellulose plates (Merck, Cat. No. 5577), 20 cm × 20 cm × 0.1 mm. Migration distance 10 cm. Visualization: iodine vapour. Eluents: 0.1 mol L^{-1} NaCl (H); ethanol–pyridine–water 1 : 1 : 1 (I).
Trp	57(L)	62	1.23	H	
4-MethylTrp	29(L)	36	1.37	H	
	42(L)	52	1.50	I	
5-MethylTrp	37(L)	46	1.45	H	
	48(L)	54	1.27	I	
6-MethylTrp	32(L)	39	1.35	H	
	47(L)	55	1.37	I	
7-MethylTrp	34(L)	41	1.34	H	
4-FluoroTrp	38(L)	44	1.28	H	
	53(L)	60	1.33	I	
5-FluoroTrp	39(L)	45	1.27	H	
	59(L)	64	1.23	I	
6-FluoroTrp	41(L)	46	1.22	H	
	61(L)	65	1.18	I	
5-HydroxyTrp	31(L)	36	1.25	H	
	41(L)	48	1.33	I	
Kynurenine	48(L)	55	1.32	H	
	43(L)	51	1.38	I	

[a] $hR_F = R_F \times 100$.
[b] $\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.
[c] Two successive developments with the same eluent.

DiopBooks

completely resolved, the D forms being the most retained.

Acidic and Basic Drugs

The enantiomers of basic β-blocking drugs can be separated on HPTLC DIOL F_{254} plates (Merck) with *N*-CBZ-Gly-L-Pro (or similar chiral agents) in the mobile phase, while the separation of the same drugs, derivatized with (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate in dichloromethane, has been performed on HPTLC-NH_2 F_{254} plates chemically bonded with *N*-(3,5-dinitrobenzoyl)-*R*-(−)-α-phenylglycine (DNBPG) and eluted with different mixtures of *n*-hexane/isopropanol.

Interesting separations of racemates with a β-aminoalcohol structure (i.e. ephedrine and norephedrine, and β-blockers) can be achieved on MCTA plates after their cyclization with phosgene to form 5-substituted oxazolidinones.

Table 4 Enantiomeric separation of *N*-alkyl, *N*-carbamyl and *N*-formyl amino acids and of dipeptides on Chiralplates

Racemate	hR_{F1}[a]	hR_{F2}	*Eluent*	*Remarks*
N-Formyl-*t*-Leu	48(+)	61(−)	A	A = methanol–water–acetic acid 50 : 50 : 200 (v/v/v).
N-Methyl-Abu	65	73	D	B = methanol–water–acetic acid 50 : 50 : 30 (v/v/v).
N-Ethyl-Abu	69	72	D	D = acetone–methanol–water 10 : 2 : 2 (v/v/v).
N-Methyl-Ala	64	70	D	M = 1 mmol L^- copper (II) acetate, 5% methanol (pH 5.8).
N-Methyl-Asp	58(L)	67(D)	B	Migration distance 13 cm; chamber saturation.
N-Methyl-Leu	49(L)	57(D)	A	Visualization; Ehrlich's reagent for *N*-carbamylTrp, iodine for
N-Methyl-Nle	68	77	D	*N*,*N*-dimethyl-Phe, and ninhydrin for the others.
N-Methyl-Nva	67	76	D	
N-Ethyl-Nva	70	74	D	
N-Methyl-Phe	50(D)	61(L)	A	
N,*N*-Dimethyl-Phe	55(D)	61(L)	B	
N-Methyl-m-Tyr	36	52	B	
N-Methyl-Val	65(L)	70(D)	B	
N-Carbamyl-Trp	44(L)	55(D)	M	
Gly-Phe	57(L)	63(D)	B	
Gly-Leu	53(L)	60(D)	B	
Gly-Ile	54(L)	61(D)	B	
Gly-Val	58(L)	62(D)	B	
Gly-Trp	48(L)	55(D)	B	
Leu-Leu	19(D, L)	26(L, D)	A	
	48(D, L)	57(L, D)	B	
Ala-Phe	21(D, L)	26(L, D)	A	
	59(D, L)	65(L, D)	B	
Met-Met	29(D, L)	33(L, D)	A	
	64(D, L)	71(L, D)	B	
Asp-Phe-OCH_3	50(L, L)	62(D, D)	A	
	50(L, D)	62(D, L)	A	

[a] $hR_F = R_F \times 100$.

Many acidic drugs (**Figure 2**) are resolved as 3,5-dinitroanilyl (DNAn) derivatives on precoated HPTLC-NH_2 F_{254} plates derivatized with *R*-(−)-1-(1-naphthyl)ethyl isocyanate (**Table 8**). Although the naphthylethyl chromophore has a high UV adsorptivity, the detection problems found on plates bonded with DNBPG were not observed.

High selectivity coefficients are obtained for underivatized acidic drugs on MCTA and diphenyl-F plates eluted with aqueous–organic solutions containing, in the latter case, a chiral macrocyclic antibiotic (vancomycin).

Other pharmaceuticals resolved include bendroflumethiazide, coumachlor, mephenytoin, oxindanac benzyl ester, warfarin, chlorowarfarin, hexobarbital, oxazepan, lorazepan, norphenylephedrine, hyoscyamine and colchicine.

Flavanones

Flavanones occur in nature and have been isolated in an optically active form. They contain only hydroxyl and methoxy groups and differ from one another in the number and/or position of such substituents (**Table 9**).

With the exception of glycosides, 5-methoxy-, 7-hydroxy- and 5-hydroxy-7-methoxyflavanone, the enantiomers of the tested compounds can be separated by at least one of the chiral phases reported in **Table 10**.

In the series of flavanones no chiral discrimination was observed on MCTA plates for racemic 2′-hydroxy-, 4′-hydroxy- and 4′-methoxyflavanone in contrast to polysubstituted compounds. Partial resolution was obtained for flavanone, 6-methoxy- and 6-hydroxyflavanone. Two successive developments with the same eluent (ethanol–water, 80 : 20 v/v) effectively improves the separation of these racemates on MCTA layers.

The addition of β-CD to the mobile phase permits separation of enantiomeric flavanone and its 2′-hydroxy-, 4′-hydroxy- and 4′-methoxy derivatives.

Albumin is able to resolve racemic polysubstituted flavanones and 2′-hydroxyflavanone. Alkaline mobile phases must be used for their separation.

Miscellaneous Compounds

The chiral NMR solvating agent 1-(9-anthryl)-2,2,2-trifluoroethanol (TFAE) has been separated by a variety of chromatographic techniques and has became a reference compound for testing new optically active selectors. For example, α values of 2.02 and 2.34

Table 5 Enantioseparation of racemic Dns-amino acids with chiral mobile phases

Dns-Amino acid	hR_{F1}[a]	hR_{F2}	α[b]	*Eluent*	*Remarks*
Abu	42(L)	47	1.22	C	Reversed-phase plates: 5 cm × 20 cm and 20 cm × 20 cm,
Ala	40(L)	47	1.33	G	KC18F, Whatman, USA. Development time 6–8 h.
Arg	55(L)	65	1.52	H	Eluents: acetonitrile–0.133 mol L^{-1} β-CD, 25 : 75 (A);
Asn	60(L)	69	1.48	H	methanol–0.163 mol L^{-1} β-CD, 35 : 65 (B);
Asp	64(L)	70	1.31	A	acetonitrile–0.151 mol L^{-1} β-CD, 30 : 70 (C);
Cit	54(L)	63	1.45	H	acetonitrile–0.133 mol L^{-1} β-CD, 20 : 80 (D);
Cys	37(L)	42	1.23	I	methanol–0.151 mol L^{-1} β-CD, 30 : 70 (E);
Gln	57(L)	66	1.46	H	acetonitrile–0.231 mol L^{-1} β-CD, 35 : 65 (F);
Glu	65(L)	72	1.38	B	methanol–0.2 mol L^{-1} β-CD, 35 : 65 (G);
His	58(L)	64	1.28	H	acetonitrile–0.2 mol L^{-1} β-CD, 20 : 80 (H);
Ile	33(L)	40	1.35	L	methanol–0.2 mol L^{-1} β-CD, 55 : 45 (I);
allo-Ile	30(L)	38	1.43	L	acetonitrile–0.2 mol L^{-1} β-CD, 32 : 68 (L);
Leu	30(L)	35	1.25	C	methanol-saturated β-CD, 60 : 40 (M);
Lys	35(L)	39	1.18	M	methanol–0.2 mol L^{-1} β-CD, 50 : 50 (N).
Met	34(L)	38	1.19	C	Aqueous solutions of β-CD also contain urea (saturated
Nle	24(L)	28	1.23	C	solution) and 3.5% sodium chloride.
Nva	32(L)	34	1.09	C	Visualization: UV_{254}.
Orn	35(L)	40	1.23	M	
Phe	35(L)	39	1.18	C	
Pro	39(L)	41	1.08	N	
Ser	41(L)	47	1.27	D	
Thr	42(L)	51	1.43	E	
Trp	43(L)	45	1.08	F	
Tyr	23(L)	26	1.17	M	
Val	36(L)	43	1.34	C	
N-Methyl-Val	24(L)	28	1.23	N	
Abu	34(L)	56	2.47	O	RP-18W/UV_{254} plates (Art. 811075, Macherey-Nagel).
Asp	68(D)	79	1.77	O	Migration distance 7 cm.
Glu	45(D)	65	2.27	O	Eluents: 5% BSA in 0.1 mol L^{-1} acetate buffer (O);
Leu	6(D)	15	2.76	P	5% BSA and 1% NaCl in 0.5 mol L^{-1} acetic acid (P);
Met	32(L)	50	2.12	Q	6% BSA in 0.1 mol L^{-1} acetate buffer (Q);
Nle	38	50	1.63	Q	7% BSA in 0.5 mol L^{-1} acetic acid (R).
Nva	25(L)	73	8.13	O	Eluents also contain 2% isopropanol.
Phe	24(L)	45	2.59	Q	Visualization: UV_{254}.
Ser	39(D)	46	1.33	R	
Thr	34(L)	43	1.46	Q	
	25(D)	32	1.41	R	
Trp	37(D)	62	2.77	O	
Val	20(L)	33	1.97	O	

[a] $hR_F = R_F \times 100$.
[b] $\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.

DropBooks

were obtained, respectively, on OPTI-TAC F_{254} (Antec) plates eluted with ethanol–water 80 : 20 (v/v) and on SIL C_{18}-50/UV_{254} layers using 6% BSA in 0.05 mol L^{-1} sodium tetraborate containing 20% isopropanol (pH 9.75) as mobile phase.

(±)-1-(9-Fluorenyl)ethanol an analogue of TFAE, was also resolved on home-made MCTA plates eluting with 2-propanol–water 80 : 20 (v/v) ($\alpha = 2.24$).

The separation of chiral compounds with restricted rotation, as in the case of binaphthyl type of substances, can be effected both on CSPs and with CMPs. The first technique requires the use of MCTA plates to resolve (±)-1,1′-binaphthyl-2,2′-diamine ($\alpha = 1.99$) while the latter involves chiral mobile phases containing BSA for the separation of (±)-1,1′-bi-2-naphthol ($\alpha = 2.15$) and (±)-binaphthyl-2,2′-diyl-hydrogen phosphate ($\alpha = 4.65$).

The enantiomeric separations of synthetic pyrethroids, such as alfamethrin and fenpropathrin, on home-made MCTA plates with ethanol–water 80 : 20 ($\alpha = 1.37$ and 1.20, respectively) should be noted since their optical antipodes have different rates of degradation and biological activity towards animals and plants.

The resolution of racemic fenoxaprop-ethyl on the above-mentioned CSP ($\alpha = 1.52$, isopropanol–water 80 : 20) in interesting since chlorophenoxyalkyl carboxylic acids and esters are widely used herbicides.

Table 6 Enantioseparation of derivatized amino acids by chiral TLC

Derivative	hR_{F1}[a]	hR_{F2}	α[b]	R_s[c]	*Plate*	*Eluent*[e]
Fmoc-Ala	89(L)	37	1.43	1.1	SIL C_{18}-50/UV_{254}	A
Fmoc-Cha[d]	17(L)	30	2.09	2.3	SIL C_{18}-50/UV_{254}	B
Fmoc-Leu	29(L)	36	1.37	1.3	SIL C_{18}-50/UV_{254}	C
Fmoc-Met	20(D)	28	1.55	1.1	SIL C_{18}-50/UV_{254}	D
Fmoc-Nle	18(L)	27	1.68	1.4	SIL C_{18}-50/UV_{254}	B
Fmoc-Nva	19(L)	27	1.57	1.6	SIL C_{18}-50/UV_{254}	B
Fmoc-Phe	41(L)	50	1.43	1.2	SIL C_{18}-50/UV_{254}	C
Fmoc-Pro	47(D)	77	3.78	1.6	SIL C_{18}-50/UV_{254}	B
	34(D)	40	1.29	2.0	MCTA	E
Fmoc-Trp	20(D)	41	2.79	2.7	SIL C_{18}-50/UV_{254}	D
Fmoc-Val	36(L)	43	1.35	2.3	SIL C_{18}-50/UV_{254}	C
DNP-Abu	59	89	5.75	4.0	RP-18W/UV_{254}	F
DNP-Cit	34	41	1.35	1.7	RP-18W/UV_{254}	G
DNP-Eth	45	61	1.94	2.0	RP-18W/UV_{254}	G
DNP-Eth(O_2)	26	35	1.53	2.0	RP-18W/UV_{254}	H
DNP-Leu	28(L)	54	3.02	3.6	RP-18W/UV_{254}	I
	24(D)	31	1.42	1.8	SIL C_{18}-50/UV_{254}	L
DNP-Met	28	61	4.02	3.3	RP-18W/UV_{254}	M
DNP-Met(O_2)	44(D)	50	1.27	2.0	RP-18W/UV_{254}	N
DNP-Met(O)	27	34	1.39	2.3	RP-18W/UV_{254}	N
DNP-Nle	31	63	3.78	5.1	RP-18W/UV_{254}	I
DNP-Nva	40	89	12.19	5.0	RP-18W/UV_{254}	O
DNP-Pip	45	56	1.56	1.3	RP-18W/UV_{254}	F
DNPy-Ala	47	53	1.27	1.6	RP-18W/UV_{254}	H
DNPy-Leu	45	70	2.85	4.0	RP-18W/UV_{254}	N
	38	43	1.23	1.5	SIL C_{18}-50/UV_{254}	L
	23	31	1.50	2.5	SIL C_{18}-50/UV_{254}	P
DNPy-Met	31	56	2.61	4.2	RP-18W/UV_{254}	Q
DNPy-Nle	25	63	5.11	5.7	RP-18W/UV_{254}	N
DNPy-Nva	21	31	1.69	2.3	RP-18W/UV_{254}	H
DNPy-Phe	28	61	4.02	5.0	RP-18W/UV_{254}	Q
	47	51	1.17	1.2	SIL C_{18}-50/UV_{254}	L
	18	26	1.60	2.0	SIL C_{18}-50/UV_{254}	P
DNPy-Trp	30	49	2.24	5.0	SIL C_{18}-50/UV_{254}	L
DNB-Leu	34(L)	51	2.02	3.0	RP-18W/UV_{254}	N
DNB-PhenylGly	30(L)	67	4.75	7.3	RP-18W/F_{254S}	N
MTH-Pro	12	16	1.39	1.3	RP-18W/UV_{254}	R
	33	37	1.19	1.0	MCTA	E
MTH-Phe	43	49	1.27	1.7	MCTA	S
MTH-Tyr	42	45	1.13	1.0	MCTA	E
PTH-Pro	13	25	2.23	2.5	MCTA	E
N-[1-(1-Naphthyl)ethyl]phthalamic acid	54(R)	58	1.17	1.6	MCTA	T
N-Benzylproline ethyl ester	19(D)	22	1.20	1.0	MCTA	U
Amethopterin	9(L)	19	2.34	2.3	RP-18W/UV_{254}	V
N-Acetyl-5-methyl-Trp	33	76	6.44	3.0	RP-18W/UV_{254}	M
N-CBZ-Trp	44(D)	88	9.33	3.0	RP-18W/UV_{254}	Z
N-*t*-BOC-Trp	16(L)	23	1.57	1.2	RP-18W/UV_{254}	V
N-*t*-BOC-*p*-nitro-Phe	20(D)	30	1.71	2.3	SIL C_{18}-50/UV_{254}	W
Ala-β-NA	68(L)	76	1.49	1.5	SIL C_{18}-50/UV_{254}	Y
	59	66	1.35	–	KC18F	J
Leu-β-NA	54(L)	64	1.50	2.0	SIL C_{18}-50/UV_{254}	L
Met-β-NA	45(L)	55	1.50	2.3	SIL C_{18}-50/UV_{254}	L
Ala-*p*-NA	12(L)	14	1.19	0.4	RP-18W/UV_{254}	K
Leu-*p*-NA	4(L)	7	1.81	1.2	RP-18W/UV_{254}	K_1
	19(L)	23	1.27	1.0	SIL C_{18}-50/UV_{254}	K_2
	56(L)	63	1.33	1.7	SIL C_{18}-50/UV_{254}	L

[a] $hR_F = R_F \times 100$.

[b] $\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.

[c] $R_s = 2 \times$ (distance between the centres of two adjacent spots)/(sum of the width of the two spots in the direction of development).

[d] Cha = β-Cyclohexylalanine.

[e] Eluents: A = 6% BSA, 23% 2-propanol, 0.1 mol L^{-1} acetate buffer; B = 5% BSA, 23% 2-propanol, 0.1 mol L^{-1} acetate buffer; C = 5% BSA, 36% 2-propanol, 0.1 mol L^{-1} acetate buffer; D = 6% BSA, 12% 2-propanol, 0.1 mol L^{-1} acetate buffer; E = 2-propanol/water 60 : 40 (v/v); F = 4% BSA, 2% 2-propanol, 0.1 mol L^{-1} acetate buffer (10°C); G = 6% BSA, 2% 2-propanol, 0.1 mol L^{-1} acetate buffer; H = 4% BSA, 1% NaCl, 2% 2-propanol, 0.5 mol L^{-1} acetic acid (10°C); I = 4% BSA, 2% 2-propanol, 0.05 mol L^{-1} phosphate buffer; L = 0.15 mol L^{-1} β-CD in a water–acetonitrile solution (80 : 20, v/v) containing 26% urea and 3% NaCl; M = 3% BSA, 2% 2-propanol, 0.05 mol L^{-1} phosphate buffer; N = 5% BSA, 2% 2-propanol, 0.1 mol L^{-1} acetate buffer; O = 4% BSA, 2% 2-propanol, 0.1 mol L^{-1} acetate buffer; P = Hydroxypropyl-β-CD (13.8 g) in water–acetonitrile–acetic acid (45 : 4 : 1, v/v/v, 100 mL); Q = 2% BSA, 2% 2-propanol, 0.1 mol L^{-1} acetate buffer; R = 9% BSA, 2% 2-propanol, 0.1 mol L^{-1} acetate buffer; S = 2-propanol–water 80 : 20 (v/v); T = ethanol–water 70 : 30 (v/v); U = 2-propanol–water 40 : 60 (v/v); V = 8% BSA, 2% 2-propanol, 0.5 mol L^{-1} acetic acid; Z = 5% BSA, 2% 2-propanol, 0.5 mol L^{-1} acetic acid; W = 6% BSA, 6% 2-propanol, 0.05 mol L^{-1} $NaHCO_3$ + 0.05 mol L^{-1} Na_2CO_3; Y = 0.1 mol L^{-1} β-CD in a water–acetonitrile solution (80 : 20, v/v) containing 20% urea and 3% NaCl; J = 0.163 mol L^{-1} β-CD in a water–methanol solution (65 : 35, v/v) containing 3.5% NaCl and saturated with urea; K = 8% BSA, 3% 2-propanol, 0.1 mol L^{-1} acetate buffer; K_1 = 6% BSA, 8% 2-propanol, 0.1 mol L^{-1} acetate buffer; K_2 = 8% BSA, 20% 2-propanol, 0.1 mol L^{-1} acetate buffer.

Table 7 Separation of enantiomeric α-hydroxycarboxylic acids on HPTLC-CHIR plates[a]

Racemate	hR_{F1}[b]	hR_{F2}	α[c]	*Eluent*[d]
Mandelic acid	36	48	1.64	A
4-Bromo-mandelic acid	33	44	1.59	B
4-Chloro-mandelic acid	35	42	1.34	B
3-Hydroxy-mandelic acid	47	59	1.62	B
4-Hydroxy-mandelic acid	45	57	1.61	C
3,4-Dihydroxy-mandelic acid	33	44	1.59	C
4-Hydroxy-3-methoxy-mandelic acid	24	33	1.55	A
2-Hydroxy-2-phenyl-propanoic acid	38	47	1.44	A
2-Hydroxy-3-phenyl-propanoic acid	39	51	1.62	A
Lactic acid	70(D)	76(L)	1.38	D
2-Hydroxy-butanoic acid	27	37	1.58	A
2-Hydroxy-3-methoxy-butanoic acid	33	46	1.73	A
2-Hydroxy-4-methylthio-butanoic acid	33	45	1.66	A
2-Hydroxy-pentanoic acid	25	39	1.91	A
2-Hydroxy-3-methyl-pentanoic acid	34	49	1.86	A
2-Hydroxy-4-methyl-pentanoic acid	35	47	1.64	A
2-Hydroxy-hexanoic acid	62(D)	69(L)	1.36	D
2-Hydroxy-octanoic acid	36	50	1.78	A
2-Hydroxy-tetradecanoic acid	34	49	1.86	A
2-Hydroxy-hexadecanoic acid	39	56	1.99	A
2-Hydroxy-docosahexanoic acid	39	56	1.99	A

[a]Migration distance, measured from concentrating zone, 13 cm; visualization: (a) the plates were dipped in $MnCl_2$–sulfuric acid heating up to 120°C for 30 min for aromatic α-hydroxycarboxylic acids; (b) the plates were dipped for 2 s in vanadium (V)–sulfuric acid solution and dried at room temperature for *c.* 45 min for aromatic and aliphatic α-hydroxycarboxylic acids.

[b]$hR_F = R_F \times 100$.

[c]$\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.

[d]Eluents: A = dichloromethane/methanol 45 : 5 (v/v); B = 0.05 mol L^{-1} KH_2PO_4 in a methanol–acetonitrile–water 50 : 50 : 200 (v/v/v) mixture; C = 0.1 mol L^{-1} LiCl in a dichloromethane–ethanol 85 : 15 (v/v) mixture; D = acetonitrile–water 3 : 2 (v/v).

The use of mobile phases containing β-CD seems to be particularly appropriate for the resolution of racemic *S*-(1-ferrocenyl-2-methylpropyl)thioethanol and *S*-(1-ferrocenylethyl)thioethanol (α = 1.43 and 1.18, respectively).

Many noncharged solutes with a carbonyl group close to the stereogenic centre can be resolved on MCTA plates (benzoin, benzoin methyl ether, 2-phenylbutyrophenone, 2- and 3-methylindanone, 2-phyenylcyclohexanone, 2-phenylcycloheptanone and 2-oxazolidone derivatives).

Quantitative Analysis of TLC-Separated Enantiomers

TLC is generally coupled with spectrophotometric methods for quantitative analysis. Quantification can be achieved by *in situ* densitometry or after extraction of solutes from the scraped layer. The evaluation of detection limits for separated enantiomers is essential because precise determinations of trace levels of D- or L-enantiomer in an excess of the other is becoming more and more important.

On Chiralplates and HPTLC-CHIR layers, densitometry can be performed after postchromatographic derivatization of compounds with ninhydrin or vanadium pentoxide. Successful separation of amino acids on Chiralplates depends on the hydrochloric acid content of the applied solution (usually a methanol–0.1 mol L^{-1} HCl 1 : 1 (v/v) mixture).

Remission–location curves of DL-α-hydroxycarboxylic acids, achieved in reflectance mode with a Shimadzu CS930 scanner or a Desaga CD60 densitometer, show that only enantiomers with high ΔR_F values ($\geqslant 0.10$) can be baseline resolved on 10 cm × 10 cm HPTLC-CHIR plates (**Figure 3**). On such plates, L-2-hydroxy-3-phenylpropionic acid spiked with 1% D enantiomer ($\Delta R_F = 0.12$) gives rise to partially resolved peaks but the D isomer is still visible. With respect to small particle size HPTLC-CHIR layers, higher R_s values have been obtained on 20 cm × 20 cm Chiralplates owing to migration distances being twice as long (α values being equal).

The remission–location curves of **Figure 4** and the calibration line for L-phenylalanine (**Figure 5**) demonstrate that quantitative determinations of L-isomer in D-phenylalanine on Chiralplates ($\Delta R_F = 0.10$) are possible in a working range of 0.04–0.4 μg/spot, that is 0.1–1%. Further determinations include 0.1% D-*t*-Leu in L-*t*-Leu ($\Delta R_F = 0.11$), 0.1% L-5,5-dimethylthiazolidine-4-carboxylic acid in the D-enantiomer ($\Delta R_F = 0.14$) and 1% D-hydroxyphenylalanine in the L-enantiomer.

The peak of 1% Dns-D-Glu in L-enantiomer is visible on 20 cm × 20 cm RP-18 plates (Merck) impregnated with a solution of 8 mol L^{-1} *N*,*N*-di-*n*-propyl-L-alanine and 4 mmol L^{-1} cupric acetate.

On 10 cm × 20 cm cellulose plates L-tryptophan spiked with 5% D-enantiomer gives rise to partially resolved peaks owing to the small ΔR_F value (0.06).

The use of MCTA allows the determination of enantiomeric mixtures in the ratios 100 : 1 and 200 : 1. (*S*)-2,2,2-Trifluoro-1-(9-anthryl)ethanol can be detected at 1% level in (*R*) enantiomer on OPTI-TAC F_{254} plates eluted with ethanol–water 80 : 20 ($\Delta R_F = 0.17$; length of run 10 cm). Baseline-resolved

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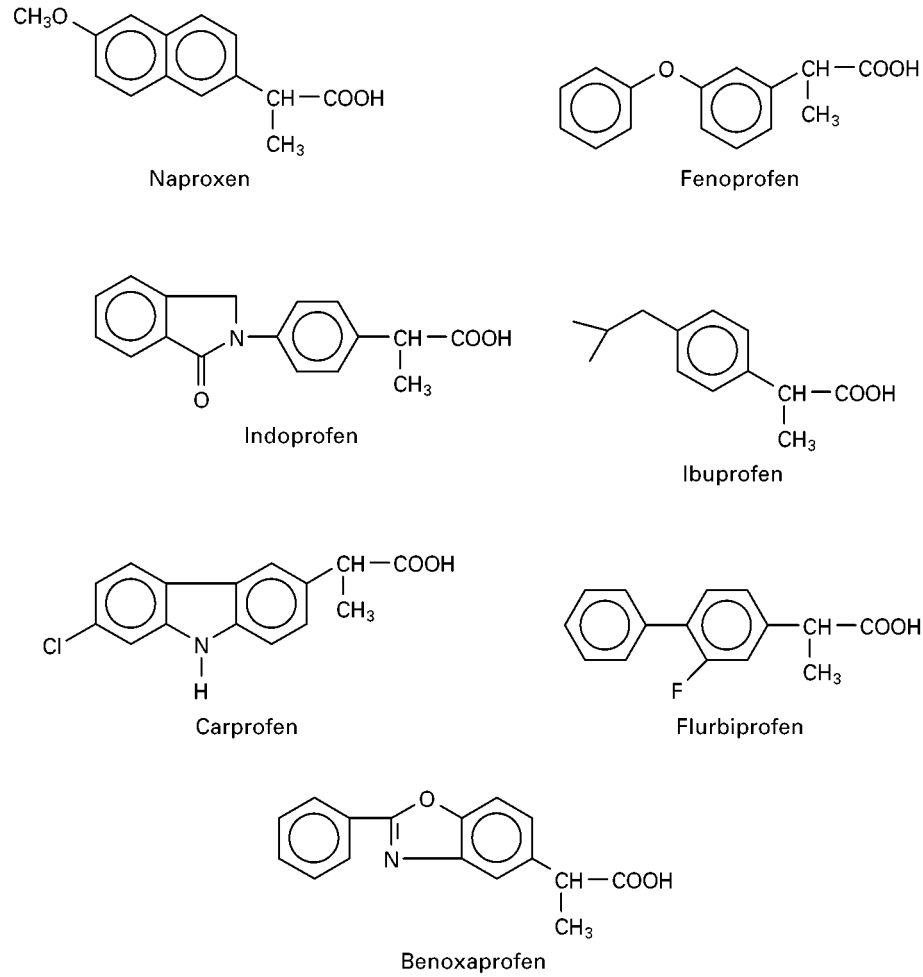


Figure 2 Structure of acidic drugs.

peaks ($\Delta R_F = 0.10$) were obtained for the two atropisomers of 1,1′-binaphthyl-2,2′-diamine on 20 cm × 20 cm home-made MCTA plates at 100 : 1 ratio. Partial resolution only was observed at a ratio of 200 : 1, but the *S* isomer is still visible (**Figure 6**).

Conclusions

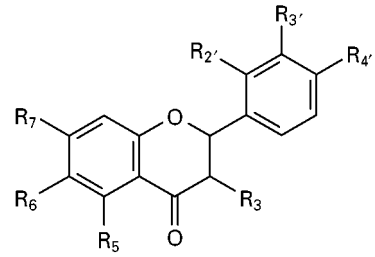
Chiral TLC plays a significant role both in economical routine analyses and in determination of optical purity of individual antipodes. Detection limits of ⩾0.1% D- or L-isomer can be currently achieved.

Table 8 Retention and resolution data for derivatized and free acidic drugs by chiral TLC

Drug	hR_{F1}[a]	hR_{F2}	α[b]	*Eluent*[c]	*Plates and remarks*
DNAn-ibuprofen	28 (S)	45 (R)	2.10	A	Precoated 10 cm × 10 cm HPTLC-NH_2 F_{254}s plates
DNAn-naproxen	15 (S)	24 (R)	1.79	A	(Altech, Deerfield, IL, USA), derivatized with (*R*)-(−)-1-
DNAn-fenoprofen	23	33	1.65	A	(1-naphthyl)ethyl isocyanate.
DNAn-flurbiprofen	23	33	1.65	A	Visualization: UV_{254} and UV_{360}.
DNAn-benoxaprofen	20	30	1.71	A	
Flurbiprofen	18	24	1.44	B	MCTA plates.
Carprofen	36	41	1.23	C	Visualization: UV.
Indoprofen	58	63	1.22	D	5 cm × 20 cm chemically-bonded diphenyl-F plates.

[a] $hR_F = R_F \times 100$.
[b] $\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.
[c] Eluents: A = *n*-hexane–isopropanol–acetonitrile 20 : 8 : 1 (v/v/v); B = ethanol–water 40 : 60 (v/v); C = isopropanol–water 60 : 40 (v/v); D = acetonitrile–0.6 mol L^{-1} NaCl–1% triethylammonium acetate buffer (pH 4.1) containing vancomycin.

Table 9 The structure of racemic flavanones



R_3	R_5	R_6	R_7	$R_{2'}$	$R_{3'}$	$R_{4'}$	*Name*
H	H	H	H	H	H	H	Flavanone
H	OCH_3	H	H	H	H	H	5-Methoxyflavanone
H	H	OH	H	H	H	H	6-Hydroxyflavanone
H	H	OCH_3	H	H	H	H	6-Methoxyflavanone
H	H	H	OH	H	H	H	7-Hydroxyflavanone
H	H	H	H	OH	H	H	2′-Hydroxyflavanone
H	H	H	H	H	H	OH	4′-Hydroxyflavanone
H	H	H	H	H	H	OCH_3	4′-Methoxyflavanone
H	OH	H	OH	H	H	H	Pinocembrin
H	OH	H	OCH_3	H	H	H	Pinocembrin-7-methylether
H	OH	H	OH	H	H	OH	Naringenin
H	OH	H	OH	H	H	OCH_3	Isosakuranetin
H	OH	H	OCH_3	H	H	OH	Sakuranetin
H	OH	H	Gl[a]	H	H	OH	Naringenin-7-glucoside
H	OH	H	Rh-Gl[b]	H	H	OH	Naringin
H	OH	H	OH	H	OH	OH	Eriodictyol
H	OH	H	OH	H	OCH_3	OH	Homoeriodictyol
H	OH	H	OH	H	OH	OCH_3	Hesperetin
OH	OH	H	OH	H	OH	OH	Taxifolin

[a]Gl = Glucoside.
[b]Rh-Gl = Rhamnosidoglucoside.

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Table 10 Retention and resolution data for racemic flavanones by chiral TLC

Racemate	hR_{F1}[a]	hR_{F2}	α[b]	R_s[c]	*Plate*	*Eluent*[d]
Flavanone (F)	16	20	1.31	1.6	SIL C_{18}-50/UV_{254}	A
	22	24	1.12	0.4	MCTA	B
6-Hydroxy-F	36	39	1.14	0.8	MCTA	B
6-Methoxy-F	24	27	1.17	0.8	MCTA	B
2′-Hydroxy-F	10	16	1.71	2.0	SIL C_{18}-50/UV_{254}	C
	19	24	1.35	1.6	SIL C_{18}-50/UV_{254}	A
4′-Hydroxy-F	38	42	1.18	1.2	SIL C_{18}-50/UV_{254}	A
4′-Methoxy-F	13	19	1.57	2.0	SIL C_{18}-50/UV_{254}	A
5,7-Dihydroxy-F	54	60	1.27	1.8	MCTA	D
4′,5,7-Trihydroxy-F	23	28	1.30	1.6	MCTA	E
5,7-Dihydroxy-4′-methoxy-F	18	21	1.21	1.3	MCTA	E
4′,5-Dihydroxy-7-methoxy-F	43	48	1.22	1.2	MCTA	D
3′,4′,5,7-Tetrahydroxy-F	26	30	1.21	1.5	MCTA	E
4′,5,7-Trihydroxy-3′-methoxy-F	23	26	1.17	0.8	MCTA	E
3′,5,7-Trihydroxy-4′-methoxy-F	23	27	1.24	1.5	MCTA	E
3,3′,4′,5,7-Pentahydroxy-F	44	48	1.17	1.3	MCTA	E

[a]$hR_F = R_F \times 100$.
[b]$\alpha = (1 \div R_{F1} - 1)/(1 \div R_{F2} - 1)$.
[c]$R_s = 2 \times$(distance between the centres of two adjacent spots)/(sum of the width of the two spots in the direction of development).
[d]Eluents: A = 0.15 mol L^{-1} β-CD aqueous solution with urea (32%) and NaCl (2%)–acetonitrile 80 : 20 (v/v), migration distance 8.5 cm; B = ethanol–water 80 : 20 (v/v), migration distance 12 cm; C = 0.05 mol L^{-1} sodium bicarbonate + 0.05 mol L^{-1} sodium carbonate solution containing 6% BSA and 12% isopropanol, migration distance 8 cm; D = ethanol–water 70 : 30 (v/v), migration distance 14 cm; E = methanol–water 80 : 20 (v/v), migration distance 16 cm.

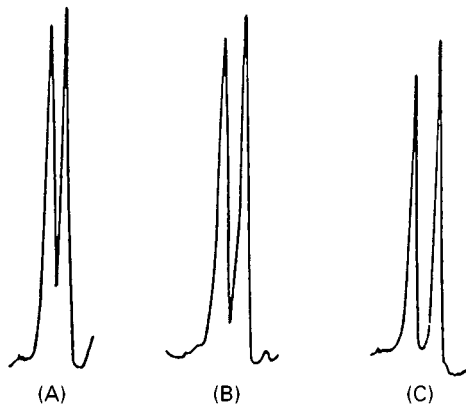

Figure 3 Remission–location curves recorded on 10 cm × 10 cm HPTLC-CHIR plates. (A) D,L-Lactic acid ($\Delta R_F = 0.05$); (B) D,L-2-hydroxybutanoic acid ($\Delta R_F = 0.10$); (C) D,L-2-hydroxyoctanoic acid ($\Delta R_F = 0.14$).

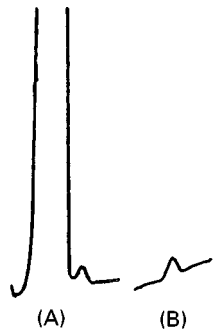

Figure 4 Remission–location curves recorded on 20 cm × 20 cm Chiralplates. (A) D-Phe spiked with 0.1% L-Phe; (B) 0.1% L-Phe.

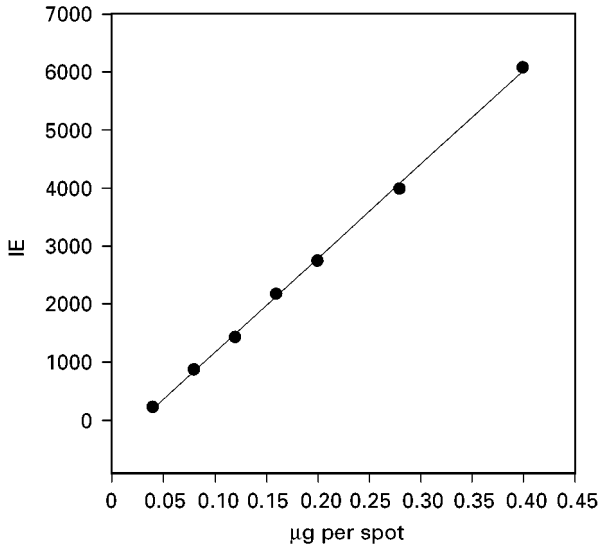

Figure 5 Calibration line for L-phenylalanine. IE, integration units; $y = -463 + 16\,349x$; $r = 0.9992$; $S_{xo} = 0.0038$ μg per spot; $\lambda = 540$ nm.

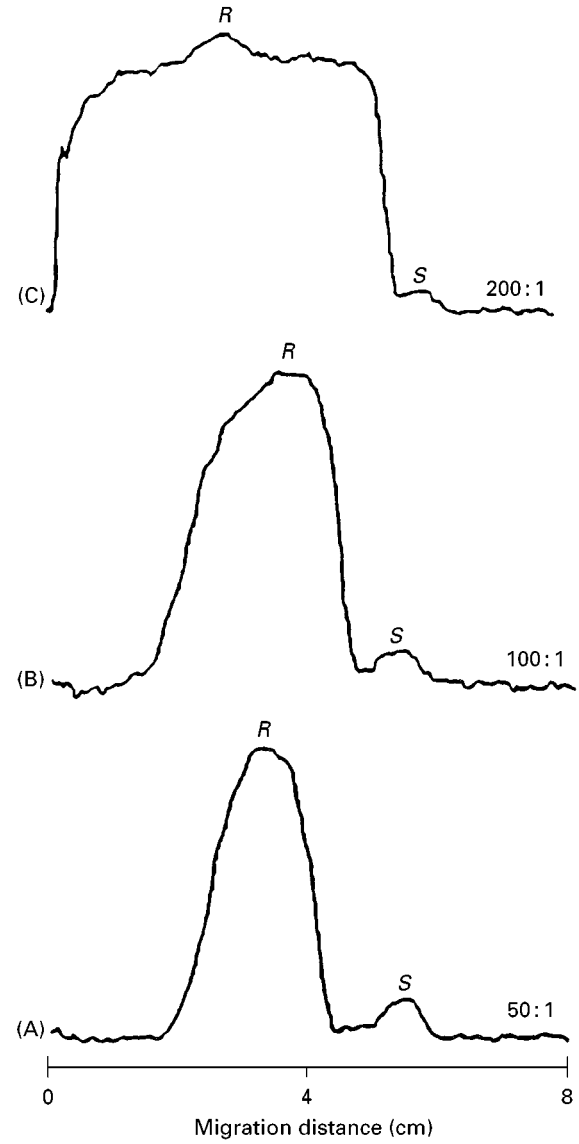

Figure 6 Densitograms of (*R*) and (*S*) - 1,1′-binaphthyl-2,2′-diamine mixtures in the ratios 50 : 1, 100 : 1 and 200 : 1 on MCTA layers, eluted with ethanol–water 80 : 20 (v/v). Migration distance 17 cm. (A) $(R) = 10$ μg, $(S) = 0.2$ μg; (B) $(R) = 20$ μg; $(S) = 0.2$ μg; (C) $(R) = 40$ μg; $(S) = 0.2$ μg.

Less work is being carried out on chiral TLC than on column chromatography, even though the two techniques may give complementary results and TLC has advantages such as low cost and easy evaluation of the tests.

Future possibilities of chiral TLC include:

1. the synthesis of enantiomeric derivatives that are easier to resolve and more sensitively detected than those so far investigated;
2. the availability of layers prepared from new cellulose derivatives and, in addition, the availability of more versatile MCTA plates using highly crystalline and homogeneously sized material;

3. more extensive application of normal-phase chromatography with a chiral mobile phase additive (DIOL plates are particularly advisable);
4. the use of eluents containing new chiral selectors in reversed-phase systems, which is the technique most widely used for enantioseparations.

See also: **II/Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Layers; Spray Reagents. **III/Amino Acids and Derivatives: Chiral Separations: Chiral Separations:** Capillary Electrophoresis; Cellulose and Cellulose Derived Phases; Chiral Derivatization; Cyclodextrins and Other Inclusion Complexation Approaches; Ion-Pair Chromatography; Ligand Exchange Chromatography; Liquid Chromatography; Molecular Imprints as Stationary Phases; Protein Stationary Phases; Supercritical Fluid Chromatography; Synthetic Multiple Interaction ('Pirkle') Stationary Phases.

Further Reading

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